^{32}P primer for 141 Vent
Human spleen DNA

Pag N __ ^{32}P 2633 (into the anchor primer)
follow P. 53 except use more ^{32}P ATP

~26% primers have ATP; 100% efficiency in labeling

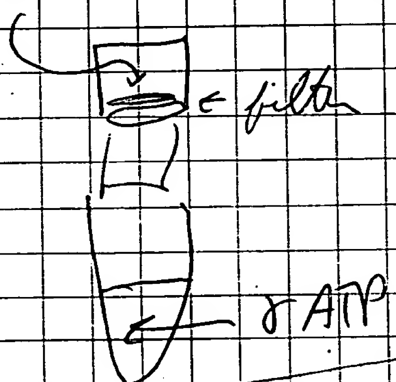
		①		(159 pm primer) ②
oligo 2633	159 μM	1 μl	✓ ✓	✓
^{32}P γ ATP 10 mCi/μl 10-21-94 (1.67 μM ATP)	6000 Ci/mmol	25 μl	✓ ✓	✓ (41.8 pm ATP)
5X Kinase buffer		6.75	✓ ✓	✓
PNK 50 u/μl		0.25 μl	✓	
		33.75		

dry down 1 kb ladder
1 μl H_2O
1 μl ^{32}P dCTP
15' 37°C
1 μl EDTA

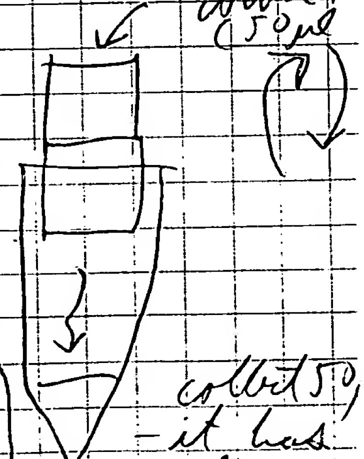
37°C 30 min → 5' 55°C → add

~~spin col same as P 54, 7, and 145, 3~~

dilute ^{32}P2633 with 100 μl H_2O (V_f = 133 now)
spin in microfuge in "micron 3"
(amicon # 42403) - after all went in, put
add 200 μl more H_2O and spin again
remove volume that did not enter filter



invert filter →



10-24-94
Had a problem - filter kept peeling back on micron 3. Maybe g force was too high on Beckman microfuge "E" model
will skip separation of free ATP.
∴ ^{32}P 2633 is diluted only 33.75 fold for
C_f = 4.71 μM

collect 50 - it has oligo

To Pag No.____

Witnessed & Understood by me, [signature]
Date 10/24/94
Invented by [signature]
Recorded by
Date 10-19-94
10/24/94